IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of	)	Attorney Docket Number: 50915
	)	
HAUER et al.	)	Confirmation No.: 6323
	)	
Serial No.: 10/031,146	)	Examiner: PAK
	)	
Filing or 371(c) Date: 01/17/2002	)	Art Unit: 1652

For: NOVEL CYTOCHROMEP450 MONOOXYGENASE AND THEIR USE FOR THE OXIDATION OF ORGANIC COMPOUNDS

Honorable Commissioner for Patents
Alexandria, Virginia 22313-1450

STATUS INQUIRY

Applicants respectfully request an update on the status of the above-referenced application.

Applicants filed Power of Attorney appointing practitioners associated with customer number 26474 on November 27, 2007, along with a Petition under 37 C.F.R. §1.137(B), and Reply under 37 C.F.R. §1.111. However, the electronic file of the above-referenced application which is kept on the USPTO's Private Pair system continues to be inaccessible to applicants' representatives.

Early clarification of the status of the application is respectfully solicited.

Please charge any shortage in fees due in connection with the filing of this paper to Deposit Account 14.1437. Please credit any excess fees to such account.

Respectfully submitted,
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Enclosure(s): Copies of papers as filed on November 27, 2007
Acknowledgement Receipt dated November 27, 2007

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	Hauer et al.	Docket No.:	50915
Serial No.:	10/031,146	Confirmation No.:	6323
Filing Date:	1/17/2002	Examiner:	PAK, YONG D
Customer No.:	26474	Art Unit:	1652

For: Novel Cytochrome P450 Monooxygenase and their Use for Oxidizing Organic Dompounds

Honorable Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

POWER OF ATTORNEY, CORRESPONDENCE ADDRESS INDICATION AND STATEMENT UNDER 37 C.F.R. §3.73(b)

I/we hereby appoint the practitioners associated with **Customer Number 26474** as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith.

Please recognize or change the correspondence address for the above-identified application to the address associated with the above-mentioned Customer Number.

BASF Aktiengesellschaft, a corporation, states that it is the assignee of the entire right, title and interest in the patent identified above by virtue of an assignment from the inventor(s) of the patent application identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel/Frame: 012725/0714.

The undersigned (whose title is supplied below) is/are authorized to act on behalf of the assignee. BASF Aktiengesellschaft

Name:	ppa. Bieller	Name:	ppa. Köster
Signature:	ppa. Bieller	Signature:	ppa. Köster
Title:	Directors	Title:	Directors
Date:	08. Nov. 2007	Date:	08. Nov. 2007

Date: FILED 11/12/07

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	Hauer et al.	Docket No.:	50915
Serial No.:	10/031,146	Confirmation No.:	6323
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For: Novel Cytochrome P450 Monooxygenase and their Use for Oxidizing Organic Dompounds

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PETITION UNDER 37 C.F.R. §1.137(B)

Sir:

The above-mentioned application became abandoned for failure to file a timely reply to a notice or action by the Office. The date of abandonment is the day after the expiration date of the period set for reply in the Office notice or action plus any extensions of time actually obtained.

Applicants hereby petition to revive this unintentionally abandoned application. The required reply to the outstanding Office Action mailed December 21, 2005, is enclosed herewith. The entire delay in filing the required reply from the due date for the required reply until the filing of a grantable petition under 37 CFR §1.137(b) was unintentional.

The petition fee of $1,540.00, as set forth in 37 CFR §1.17(m), is also enclosed. Since this application was filed after June 8, 1995, no Terminal Disclaimer is required. Please charge any shortage in fees due in connection with the filing of this paper, including any shortage in Extension of Time fees, to Deposit Account 14.1437. Please credit any excess fees to such account.

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FILED
Date: 11/27/07

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:	Hauer et al.	Docket No.:	50915
Serial No.:	10/031,146	Confirmation No.:	6323
Filing Date:	1/17/2002	Examiner:	PAK, YONG D
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For: Novel Cytochrome P450 Monooxygenase and their Use for Oxidizing Organic Dompounds

Honorable Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

REPLY UNDER 37 C.F.R. §1.111

Sir:

This is a reply to the non-final Office Action of December 21, 2005. Please enter and consider the following amendments and remarks.

Please charge any shortage in fees due in connection with the filing of this paper, including any shortage in Extension of Time fees, to Deposit Account 14.1437. Please credit any excess fees to such account.

Table of Contents

AMENDMENTS

Amendments to the Claims

Please amend the claims according to the following listing of the claims.

Listing of the claims

1. (withdrawn) A cytochrome P450 monooxygenase which is capable of at least one of the following reactions:

   a) oxidation of optionally substituted N-, O- or S-heterocyclic mono- or polynuclear aromatic compounds;
   b) oxidation of optionally substituted mono- or polynuclear aromatics;
   c) oxidation of straight-chain or branched alkanes and alkenes;
   d) oxidation of optionally substituted cycloalkanes and cycloalkenes;

   where the monooxygenase is derived from cytochrome P450 monooxygenase BM-3 from Bacillus megaterium having an amino acid sequence according to SEQ ID NO:2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43. 48-52. 67-70, 330-335, 352-356, 73-82 and 86-88; except the single mutant Phe87Val.

2. (withdrawn) A monooxygenase as claimed in claim 1, which has at least one functional mutation in at least one of the sequence regions 73-82, 86-88 and 172-224.

3. (withdrawn) A monooxygenase as claimed in claim 1,

which has at least one of the following mono- or polyamino acid substitutions:

a) Phe87Val, Leul88Gln; or
b) Phe87Val, Leu188Gln, Ala74Gly;

and functional equivalents thereof which are capable of at least one of the above oxidation reactions.

4. (withdrawn) A nucleic acid sequence coding for a monooxygenase according to claim 1.

5. (withdrawn) An expression construct comprising, under the genetic control of regulatory nucleic acid sequences, a coding sequence which comprises a nucleic acid sequence according to claim 4.

6. (withdrawn) A vector comprising at least one expression construct according to claim 5.

7. (withdrawn) A recombinant microorganism transformed by at least one vector as claimed in claim 6.

8. (withdrawn) A microorganism as claimed in claim 7, selected from bacteria of the genus Escherichia.

9. (currently amended) A process for the microbiological oxidation of an N- or S-heterocyclic mono- or polynuclear aromatic compound which comprises

a1) culturing a recombinant microorganism which expresses a cytochrome P450 monooxygenase of

bacterial origin in a culture medium, in the presence of an exogenous or intermediately formed substrate; or

a2) incubating a substrate-containing reaction medium with a cytochrome P450 monooxygenase of bacterial origin; and

b) isolating the oxidation product formed or a secondary product thereof from the medium, and

wherein the monooxygenase is derived from cytochrome P450 monooxygenase BM-3 from *Bacillus megaterium* having ~~an~~ the amino acid sequence according to SEQ ID NO: 2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43, 48 - 52, 67-70, 330-335, 352-356, 73-82 and 86-88.

10. (currently amended) A process as claimed in claim 9, wherein the exogenous or intermediately formed substrate of claim 9, alternative a1), or the substrate contained in the reaction medium of claim 9, alternative a2) is selected from optionally substituted N- or S-heterocyclic mono- or polynuclear aromatic compounds.

11. (canceled)

12. (previously presented) A process as claimed in claim 9, where the mutant has one of the following mono- or polyamino acid substitutions:

a) Phe87Val;

b) Phe87Val, and Leu188Gln;

c) Phe87Val, and Leu188Gln, and Ala74Gly.

13. (withdrawn) A process for microbiological oxidation of optionally substituted mono- or polynuclear aromatics, straight-chain or branched alkanes or alkenes, or optionally substituted cycloalkanes or cycloalkenes, which comprises

a1) culturing a recombinant cytochrome P450-producing microorganism as claimed in claim 7 in a culture medium, in the presence of an exogenous or intermediately formed substrate; or

a2) incubating a substrate-containing reaction medium with a cytochrome P450 monooxygenase derived from cytochrome P450 monooxygenase BM-3 from *Bacillus megaterium* having an amino acid sequence according to SEQ ID NO:2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43, 48-52,67-70, 330-335, 352-356, 73-82 and 86-88; and

b) isolating the oxidation product formed or a secondary product thereof from the medium;

where the monooxygenase mutant Phe87Val is not excluded.

14. (withdrawn) A process as claimed in claim 13, wherein the exogenous or intermediately formed substrate is selected from:

a) optionally substituted mono- or polynuclear

aromatics;

b) straight-chain or branched alkanes and alkenes;

c) optionally substituted cycloalkanes and cycloalkenes.

15. (canceled)

16. (withdrawn) A process as claimed in claim 13, where the cytochromeP450 monooxygenase has at least one of the following mono- or polyamino acid substitutions:

a) Phe87Val;

b) Phe87Val, Leu188Gln; or

c) Phe87Val, Leu188Gln, Ala74Gly.

17. (previously presented) A process as claimed in claim 9, wherein, as exogenous substrate, at least one compound selected from unsubstituted or substituted N-, O- or S-heterocyclic mono- or polynuclear aromatic compounds is added to a medium and the oxidation is carried out by enzymatic reaction of the substrate-containing medium in the presence of oxygen at a temperature of approximately 20 to 40°C and a pH of approximately 6 to 9, where the substrate-containing medium additionally contains an approximately 10- to 100-fold molar excess of reduction equivalents based on the substrate.

18. (previously presented) A process as claimed in claim 17, wherein, as exogenous substrate, a compound selected from indole, 1-methylindole, acridine, 6-methyl- or 8-methylquinoline, quinoline and quinaldine

is employed.

19. (withdrawn) A process for the microbiological production of indigo and/or indixubin, which comprises

a1) culturing a recombinant microorganism which produces an indole-oxidizing cytochrome P450 in a culture medium, in the presence of exogenous or intermediately formed indole; or

a2) incubating an indole-containing reaction medium with an indole-oxidizing cytochrome P450 monooxygenase; and

b) isolating the oxidation product formed or a secondary product thereof from the medium.

20. (withdrawn) A process as claimed in claim 19, wherein the indigo and/or indirubin obtained, which was produced by oxidation of intermediately formed indole, is isolated from the medium.

21. (withdrawn) A process as claimed in claim 20, wherein the indole oxidation is carried out by culturing the microorganisms in the presence of oxygen at a culturing temperature of approximately 20 to 40°C and a pH of approximately 6 to 9.

22. (withdrawn) A process as claimed in claim 20, where the monooxygenase is derived from cytochrome P450 monooxygenase BM-3 from *Bacillus megaterium* having an amino acid sequence according to SEQ ID NO:2, which has at least one functional mutation in at least one of the amino acid sequence regions 172-224, 39-43, 48-

52, 67-70, 330-335- 352-356, 73-82 and 86-88, including the substitution Phe87Val.

23. (withdrawn) A process as claimed in claim 22, where the monooxygenase has at least one of the following mono- or polyamino acid substitutions:

a) Phe87Val;
b) Phe87Val, Leu188Gln; or
c) Phe87Val, Leu188Gln, Ala74Gly.

24. (withdrawn) A bioreactor comprising the cytochrome P450 monooxygenase as claimed in claim 1 or a recombinant microorganism transformed by a vector comprising an expression construct comprising a nucleic acid sequence coding for the cytochrome P450 monooxygenase of claim 1 in immobilized form.

25. (canceled)

26. (canceled)

REMARKS

Regarding the Prosecution History:

Applicants are thankful for the Examiner's diligent efforts to advance this application to allowance and are pleased to have this opportunity to address the Examiner's remaining concerns. Upon careful review of the remarks presented in this reply, the Examiner will agree that the claimed invention is patentable and that this application is in good condition for allowance.

In the non-final Office Action of December 21, 2005, the Examiner objected to claim 17, alleging that it depends from a non-elected claim. Please enter and consider the amendments presented in the Appeal Brief filed January 25, 2005. In that Brief, applicants amended claims 12, 17 and 18 to overcome the Examiner's previous objections.

In the non-final Office Action of December 21, 2005, the Examiner rejected:

I. Claims 9 – 10, 12 and 17 – 18 under 35 U.S.C §U.S.C. §112, second paragraph;

II. Claims 9 – 10, 12 and 17 – 18 under 35 U.S.C §112, first paragraph; and

III. Claims 9 – 10, 12 and 17 under 35 U.S.C §102(b) and (e) over Wong et al. (GB 2 294 692).

Regarding the Claim Amendments presented in this reply:

The amendments to the claims add no new matter. The amendment to claim 9 merely adopts the Examiner's suggestion to put the claim in better form. The amendment to claim 10 finds support in claim 9, from which claim 10 depends.

Regarding Rejection I:

The Examiner should withdraw the rejections of claims 9 – 10, 12 and 17 – 18 under 35 U.S.C §U.S.C. §112, second paragraph.

The phrase "derived from" in claim 9 is clear in the context of the specification. The paragraph from line 15 to line 26 on page 3 actually defines the meaning of "derived" in this context as "mutated." The discussion on page 3, line 28 to page 4, line 15 of the specification elaborates on and even exemplifies the concept of "derived monooxygenases." Morover, the discussion on page 4, line 17 to page 5, line 9 extends the definition to "functional equivalents." The specification, therefore, provides a skilled artisan with a clear understanding of the term "derived monooxygenase."

The phrase "having an amino acid sequence according to SEQ ID NO: 2" in claim 9 has been amended, rendering the rejection moot. Applicants note, however, that contrary to the Examiner's suggestion in the second paragraph of page 5 of the Office action mailed December 21, 2005, the polypeptide does not have the amino acid sequence of SEQ ID NO:2, but rather differs from SEQ ID NO:2 by having at least one functional mutation in at least one of the amino acid sequence regions specified in claim 9, i.e., 172-224, 39-43, 48-52, 67-70, 330-335, 352-356, 73-82 and 86-88. Thus, claim 9 relates to an amino acid sequence which carries function mutations and is, therefore, derived from SEQ ID NO:2, but is not identical to it.

Regarding the phrase "exogenous or intermediately formed substrate" in claim 10,[1] claim 10 has been amended so as to render this rejection moot. This amendment makes it clear that claim 10 does not refer to intermediately formed substrates in case the corresponding process of claim 9 does not refer to such intermediately formed substrates. Applicants note that the Examiner has no legal basis to demand process steps for forming intermediate substrates in claim 10, because Applicants are entitled to a scope of protection which adequately reflects their contribution to the art. The process for microbiological oxidation based on cytochrome P450 monooxygenase works just as well with exogenously added substrate as with intermediately formed substrate. It would,

[1] The Examiner's reference to claim 12 on page 5 of the Office action seems to be a typographical error, since claim 12 does not include this phrase.

therefore, not be justified to exclude from the scope of protection those process which involve intermediately formed substrates. The term "intermediately formed" substrate itself is clear to a skilled artisan and denotes a compound which is not added as pre-made, exogenous substrate, but – starting from one or more precursor molecules – is formed by a microorganism (whereby the precursor molecule(s) in turn may be intermediately formed or exogenously added compounds). In this context, the Examiner is directed to page 12, lines 37 – 40, which exemplify the difference between intermediately formed and exogenously added substrate.

The phrase "secondary product thereof" in claim 9[2] would be clear to a skilled artisan. An oxidation product according to claim 9, step b) can further be modified within a recombinant microorganism or a reaction medium and therefore give rise to secondary products thereof. This concept, which is already clear to a skilled artisan, is even briefly exemplified on page 2, lines 40 – 42 of the present specification. This portion of the specification mentions the possibility of further converting the immediate reaction product in the context of a non-enzymatic subsequent or side reaction.

The phrase "functional mutation" in claim 9 is defined in on page 3, lines 20 – 21 as "promoting the oxidation of novel organic substrates." In the context of claim 9, the phrase is also clear that the substrates are N-, O-, or S- heterocyclic mono- or polynuclear aromatic compounds. The specification provides ample information how to identify said functional mutations. The paragraph from page 3, line 41 to page 4, line 2 exemplifies that functional mutations can be obtained by amino acid substitutions. The subsequent paragraph on page 4, lines 4 – 15 provides a link between the phrases "functional mutation" and "functional equivalent," and the concept of functional equivalents is extensively described on page 4, line 17 through page 4, line 9. In particular, functional equivalents are again referred to as showing a "modified substrate profile" (page 4, line 31). Thus, the specification provides a skilled artisan with a clear understanding of the concept of functional mutations.

[2] The Examiner's reference to claim 12 on page 6 of the Office action seems to be a typographical error, since claim 12 does not include this phrase.

Regarding Rejection II:

The Examiner should withdraw the rejection of claims 9 – 10, 12 and 17 – 18 under 35 U.S.C §112, first paragraph.

According to the MPEP, "[d]escription of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces" and as such, a single species may be enough to identify the entire genus (see MPEP 2163.II.A.3.a.ii.).

"The 'written description' requirement states that the patentee must describe the invention; it does not state that every invention must be described in the same way. As each field evolves, the balance also evolves between what is known and what is added by each inventive contribution" (418 F.3d 1358; 2005). The instant Specification, even with only one example, provides a complete written description. One of ordinary skill in the art would not require undue experimentation to create the instant invention because the art at the time of filing would allow said creation.

Moreover, the Examiner is inappropriately requiring conclusive evidence, whereas applicants have provided enough information in the disclosure for one of ordinary skill in the art to practice the invention. According to accepted principles of patent practice applicants are not merely entitled to the literally disclosed invention. If that were the case, then the scope of protection would always be limited to the disclosed examples. However, applicants are rather entitled to the whole range of embodiments, which are made available by their invention without undue experimentation.

Applicants created functionally mutated P450 BM-3 proteins, which – contrary to native P450 BM-3 – have the ability to produce blue indigo-containing pigment (see: experimental result 1, page 18), verified the produced pigment as indigo (see: experimental result 2, page 19) and used one of the mutated P450 MB-3 proteins in a process for producing indigo from indole (experimental result 3, pages 19 – 20). Moreover, Applicants used a mutated P450 BM-3 protein for the oxidation of 8-methylquinoline, a hetero-aromatic compound (Example 7b, page 22). Thus, Applicants have properly shown that the claimed invention can be practiced. It would be within the skill of a person of ordinary skill in the art to apply the invention to the embodiments

within the scope of the present claims, the rejection under 35 U.S.C §112, first paragraph is in error and should be withdrawn.

Regarding Rejection III:

The Examiner should withdraw the rejection of claims 9 – 10, 12 and 17 under 35 U.S.C §102(b) and (e) over Wong et al. (GB 2 294 692). The Examiner stated that

> Wong et al. … discloses a method of oxidizing N-heterocyclic polynuclear aromatic compound [*sic*] with a modified cytochrom P450 monooxygenase having a mutation corresponding to reside 87 of SEQ ID NO:2. (abstract and pages 4 and 14). Since applicants do not place any limitation on the structure of the monooxygenase derived from SEQ ID NO:2, Examiner takes the position that the monooxygenase of Wong et al. is a cytochrome P450 monooxygenase that is 'derived from *Bacillus megaterium*.[3]

However, neither the specifically indicated abstract or the pages 4 or 14 nor any other part of GB 2 294 692 mentions N-heterocyclic polynuclear aromatic compounds as substrates. To the contrary, the second complete paragraph on page 4 of GB 2 294 692 makes clear the range of substrates of GB 2 294 692.

> According to another aspect of the present invention a mutant of the mono-oxygenase cytochrome P-450cam is provided in which the tyrosine residue at position 96 and/or the cysteine residue at position 334 is replaced by another amino acid residue, which mutant has the property of catalysing the oxidation of any one of the following: polycyclic aromatic hydrocarbons, linear or branched alkanes, diphenyl and biphenyl compounds including halogenated variants of such compounds and halogenated hydrocarbons.[4]

GB 2 294 692 only mentions one heterocyclic compound in Scheme 1, but makes clear in

[3] Page 15, lines 12 – 18 of the Office action mailed December 21, 2005.
[4] Page 4, second complete paragraph of GB 2 294 692.

the specification that its use is limited to that of a protection group, not that of a substrate for the oxidation (page 7, last paragraph):

> Examples of monofunctionalised hydrocarbons are cyclohexyl, cyclopentyl and alkyl derivatives (Scheme 1). The oxidation products of *these* compounds are valuable starting materials for organic synthesis, particularly when produced in a homochiral form. A range of aromatic *protecting groups* are envisaged, e.g. benzyl or naphtyl ether and benzoyl or naphthoyl esters and amids (Scheme 1). Of interest are also benzoxazole groups as carboxyl *protecting groups* and N-benyl oxazolidine groups as aldehyde *protecting groups*. Both can be easily cleaved after the enzymatic oxidation and have previously been described in the literature for the microbial oxidations of aldehydes and acids.[5]

In GB 2 294 692, the heterocyclic compounds serve as protection groups, they are added before the oxidation and removed after the oxidation without affecting the oxidized product of interest. In the present invention, however, the heterocyclic compound is an integral part of the oxidized product, in other words: it actually is the product of interest. Any attempt to remove the heterocyclic portion would destroy the product of the inventive oxidation process.

The same considerations apply with regard to US 6,110,074, which is nearly identical to GB 2 294 692.

For at least these reason, it is respectfully submitted that the present rejection is in error and should be withdrawn.

In Conclusion:

The present application is in condition for allowance. Again, applicants are thankful for the Examiner's diligent efforts to advance this application to allowance, and request favorable action in this matter. In order to facilitate the resolution of any issues

[5] Page 7, last paragraph of GB 2 294 692 (emphasis added).

or questions presented by this paper, the Examiner is welcome to contact the undersigned by phone to further the discussion.

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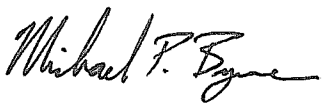

Michael P. Byrne
Registration No.: 54,015

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The USPTO has received your submission at **16:37:51** Eastern Time on **27-NOV-2007** .

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eFiled Application Information

EFS ID	2514359
Application Number	10031146
Confirmation Number	6323
Title	Novel cytochrome p450 monooxygenases and their use for oxidizing organic compounds
First Named Inventor	Bernhard Hauer
Customer Number or Correspondence Address	Keil & Weinkauf 1350 Connecticut Avenue NW Washington DC 20036 US
Filed By	Tracy Wesley Druce/Slawek Mosiolek
Attorney Docket Number	50915
Filing Date	17-JAN-2002
Receipt Date	27-NOV-2007
Application Type	U.S. National Stage under 35 USC 371

Application Details

Submitted Files	Page Count	Document Description	File Size	Warnings
10031146-071108-Power_of_Attorney.pdf	1	Power of Attorney	55075 bytes	PASS
10031146-071127-Petition-A.pdf	16		626183 bytes	PASS

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New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see

37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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